

Wells were coated with 100 µl of vaccinia stock (2.5 x 10^8 pfu per ml) diluted 1:100 in sodium bicarbonate buffer for 1 hour at 37°C. After washing three times with 0.05 % (v:v) PBS/Tween 20, the plate was blocked with 200 µl of 10% bovine serum albumin in PBS/Tween 20 for 1 hr. The plate was again washed and 100 ul of a two-fold dilution series of murine serum was added to triplicate wells for 1 hour at 37°C. After subsequent washing, 100 ul of goat-anti-mouse HRP conjugated antibody diluted 1:1000 was added to each well for 1 hour at 37°C. The plate was washed again as before and 100ul of Sure Blue (TM) TMB peroxidase substrate (KPL, Gaithersburg, MD) was added. After allowing 5 min for color development, 100 µl of Sure Blue (TM) stop solution was added to each well and the plate was read at 450 nm. The cut off point was defined as the mean of the data points shown for normal mouse serum + 3 standard deviations.

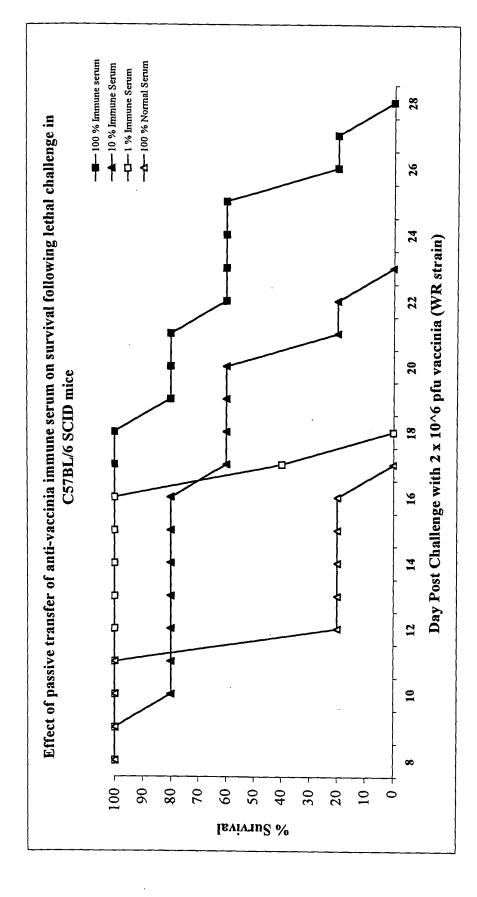


Fig. 2

1																			- Sect	ion 1
L1R-AAA48076 M1R-AAA60821 (1) MGAAASIQTTVNTLSERISSKLEQEANASAQTKCDIEIGNFYIRQNHGCNLTVK M1R-P33040 (1) MGAAASIQTTVNTLSERISSKLEQEANASAQTKCDIEIGNFYIRQNHGCNLTVK (1) MGAAASIQTTVNTLSERISSKLEQEANASAQTKCDIEIGNFYIRQNHGCNLTVK (1) MGAAASIQTTVNTLSERISSKLEQEANASAQTKCDIEIGNFYIRQNHGCNLTVK Section 2 (55) 55 60 70 80 90 108 L1R-P07612 (55) NMCSADADAQLDAVLSAATETYSGLTPEQKAYVPAMFTAALNIQTSVNTVVRDF M1R-AAA48076 (55) NMCSADADAQLDAVLSAATETYSGLTPEQKAYVPAMFTAALNIQTSVNTVVRDF M1R-P33040 (55) NMCSADADAQLDAVLSAATETYSGLTPEQKAYVPAMFTAALNIQTSVNTVVRDF Consensus (55) NMCSADADAQLDAVLSAATETYSGLTPEQKAYVPAMFTAALNIQTSVNTVVRDF Section 3 (109) 109 1,20 ,130 ,140 ,150 162 L1R-P07612 (109) ENYVKQTCNSSAVVDNKLKIQNVIIDECYGAPGSPTNLEFINTGSSKGNCAIKA M1R-P33040 (109) ENYVKQTCNSSAVVDNKLKIQNVIIDECYGAPGSPTNLEFINTGSSKGNCAIKA Consensus (109) ENYVKQTCNSSAVVDNKLKIQNVIIDECYGAPGSPTNLEFINTGSSKGNCAIKA Consensus (109) ENYVKQTCNSSAVVDNKLKIQNVIIDECYGAPGSPTNLEFINTGSSKGNCAIKA M1R-P33040 (109) ENYVKQTCNSSAVVDNKLKIQNVIIDECYGAPGSPTNLEFINTGSSKGNCAIKA Consensus (109) ENYVKQTCNSSAVVDNKLKIQNVIIDECYGAPGSPTNLEFINTGSSKGNCAIKA M1R-P30404 (109) ENYVKQTCNSSAVVDNKLKIQNVIIDECYGAPGSPTNLEFINTGSSKGNCAIKA Consensus (109) ENYVKQTCNSSAVVDNKLKIQNVIIDECYGAPGSPTNLEFINTGSSKGNCAIKA CONSENSATIONACAGAGAGATA Section 2 L1R-P07612 (107)		(1)	1		,10			20)			30			f	40		_		_ 54
M1R-AAA60821 (1) MGAAASIQTTVNTLSERISSKLEQEANASAQTKCDIEIGNFYIRQNHGCNLTVK	L1R-P07612			ASI	TTT	NTL	SER	ISS	KLE	QEA	NAS	QAS	TKC	DI	EIG	NFY	IRQ	NHO	GCNL	TVK
M1R-P33040	L1R-AAA48076	(1)	MGAA	ASI	/TTC	NTL	SER	ISS	KLE	QEA	NAS	ΙAQ	TKC	DIE	EIG	NFY	IRQ	NHO	GCNL	TVK
Consensus	M1R-AAA60821	(1)	MGAA	ASI	TTT	NTL	SER	ISS	KLE	QEA	NAS	QA	TKC	DIE	EIG	NFY	IRQ	NHO	GCNL	TVK
Section 2 Section 3 Section 4 Section 5 Section 6 Section 6 Section 5 Section 6 Sect	M1R-P33040	(1)	MGAA	ASI)TTV	NTL	SER	ISS	KLE	QEA	NAS	QA	TKC	DIE	EIGI	NFY	IRQ	NHO	GCNL	TVK
(55) 55	Consensus	(1)	MGAA	ASI	JTTV	ITUV	SER	ISS	KLE	QEA	NAS	ΩA	TKC	DIE	EIGI	MFY	IRQ	NHO	GCNL	TVK
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(109) 109	Consensus	(55)	NMCS	ADAI	DAQI	DAV	LSA	ATE'	TY S	GLI	PEÇ)KA	YVF	AMI	TA	ALN	IQT	SVI	VVTV	RDF
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M1R-AAA60821 (217) KLILANKENVHWTTYMDTFFRTSPMVIATTDIQN	L1R-P07612	(217)	KLIL	ANK	ENVI	TTWE	YMD	TFF	RTS	PMV	'IAI	TD	MQN							
	L1R-AAA48076	(217)	KLIL	ANK	ENVI	TTWE	YMD	TFF	RTS	PMV	TAI	TD	MQN	•						
M1R-P33040 (217) KLILANKENVHWTTYMDTFFRTSPMVIATTDIQN																				
······································	M1R-P33040	(217)	KLIL	ANK	ENVI	TTWI	'YMD	TFF	RTS	PMV	rai	TD.	IQN	•						

FIGURE 3.

Consensus (217) KLILANKENVHWTTYMDTFFRTSPMVIATTDMQN

										<u></u>	Section 1	1
	(1)	1		10		20		,30		40		54
A27L-P11258											ETLKQRLTN	
A27L-P20535											ETLKQRLTN	
A30L-P33816											EETLKQRLTN	
A30L-AAA60882											EETLKQRLTN	
Consensus	(1)	MDGT	LFPGD	DDLAI	PATE	FFSI	KAAKK	PEAKRI	EAIVKA	DGDDNI	ETLKQRLTN	
											——— Section 2	
	(55)		_60		.70				.90			<u>80</u>
											KKIDVQTGRR	
A27L-P20535	(55)	EKKI	TNVTT	KFEQI	EKCC	KRND	EVLFR	LENHAI	ETLRAA	MISLA	KIDVQTGRR	P
											KKIDVQTGRR	
											KIDVQTGRR	
Consensus	(55)	EKKI	TTVNT	KFEQI	EKCC	KRNI	DVLFR	LENHAI	ETLRAA	MISLA	KIDVQTGRR	
											Section 3	3
(1	109)	1109										
A27L-P11258 (1	109)	ΥE										
A27L-P20535 (1	109)	YE										
A30L-P33816 (1	109)	YE										

A30L-AAA60882 (109) YE Consensus (109) YE

							Section 1
	(1)	1	,10	,20	,30	,40	54
A33R-H42520				SATVYGDKI		GLCIRISMVIS	LLSMITMSAF
A33R-AAA48160	(1)	MMTPENI	EEQTSVF	SATVYGDKI	QGKNKRKRVI	GLCIRISMVIS	LLSMITMSAF
A36R-P33850						GICIRISMVIS	
A36R-AAA60888						GICIRISMVIS	
Consensus	(1)	MMTPENI	DEEQTSVF	SATVYGDKI	QGKNKRKRVI	GICIRISMVIS	
							Section 2
	(55)			,70	.80	90	108
A33R-H42520						'ASSTTQYDHKE	
A33R-AAA48160						ASSTTQYDHKE	
A36R-P33850						ASSTTQYKHQE	
A36R-AAA60888	(55)	LIVRLNO	QCMSANEA	AITD-ATAV	AAALSTHRKV	ASSTTQYKHQE	SCNGLYYQGS
Consensus	(55)	LIVRLNÇ	QCMSANEA	AITDAAVAV	AAASSTHRKV	'ASSTTQYKHQE	SCNGLYYQGS ——— Section 3
	(400)	400	400	404		450	
	(109)		,120	13(162
						TWLIDYVEDTW	
						TWLIDYVEDTW	
						TWLIDYVEDTW TWLIDYVEDTW	
						TWLIDYVEDTW	
Consensus	(100)	C1111111111111111111111111111111111111	JIQBI SDA	RANCTIBOL			Section 4
	(163)	163	170	185	•		
433P-H42520				KYFCVKTMN			
A33R-AAA48160							
				KYFCVKTMN			
A36R-AAA60888							
				KYFCVKTMN			
Consensus	(103)	TODION	PAPAFAK	.KIFC V KIMD			

				·		Section 1
(1)	1 1	0	20	30	40	55
D8L-P04195 (1)	MPOOLSPINI	ETKKAISNA		ESKPTTIQNT	GKLVRINFK	GGYISGG
D8L-AAA48107 (1)	MPOOLSPINI	ETKKAISNA	RLKPLDIHYN	ESKPTTIQNT	GKLVRINFK	GGYISGG
F8L-P33065 (1)	MSOOLSPINI	ETKKAISNA	RLKPLNIHYN	ESKPTTIQNT	GKLVRINFK	GGYLSGG
F8L-AAA60846 (1)	MSOOLSPINI	ETKKAISNA	RLKPLNIHYN	ESKPTTIQNI	GKLVRINFK	GGYLSGG
Consensus (1)	MSOOLSPINI	ETKKAISNA	RLKPLNIHYN	ESKPTTIQNI	GKLVRINFK	GGYISGG
Conscisce (1)						Section 2
(56)	56	,70	.80	.90	.100	110
D8L-P04195 (56)	FLDNEYVLSS			YKYSGEINLV	HWNKKKYSS	YEEAKKH
D8L-AAA48107 (56)	FLDNEVVLS	SLHIYWGKED	DYGSNHLIDV	YKYSGEINLV	HWNKKKYSS	YEEAKKH
F8L-P33065 (56)	FLPNEYVLS	SLHIYWGKED	DYGSNHLIDV	YKYSGEINLV	HWNKKKYSS	YEEAKKH
F8L-AAA60846 (56)	FLONEYVLS	SLHIYWGKED	DYGSNHLIDV	YKYSGEINLV	HWNKKKYSS	YEEAKKH
Consensus (56)	FLONEYVLS	SLHTYWGKED	DYGSNHLIDV	YKYSGEINLV	HWNKKKYSS	YEEAKKH
Consensus (66)	I BI NBI (BO.					- Section 3
(111)	111	120	,130	,140	.150	165
D8L-P04195 (111)					FDSVFYLDN	LLPSKLD
D8L-AAA48107 (111)	DDGLTTTST	FLOVSDHKNV	YFOKIVNOLD	SIRSANTSA	FDSVFYLDN	LLPSTLD
F8L-P33065 (111)	DDGLTTTST	FLOVSDHKNI	YFOKIVNOLI	SIRTANTSA	FDSVFYLDN	LLPSKLD
F8L-AAA60846 (111)	DDGLIIISI	FI OVSDHKNI	/YFOKIVNOLI	SIRTANTSA	FDSVFYLDN	LLPSKLD
Consensus (111)	DDGLTITST	FLOVSDHKNV	/YFOKIVNOLI	SIRSANTSA	FDSVFYLDN	LLPSKLD
Consensus (111)	DDGHIIIG					Section 4
(166)	166	,180	.190	200	210	220
D8L-P04195 (166)	VETYLGTTI	NHSADAVWI	FPTPINIHSI	OLSKERTLLS	SSNHDGKPH	YITENYR
DRI -AAA48107 (166)	VETYLGTTI	KHSADAVWI	FPTPINIHSI	OOLSKFRTLLS	SSSNHDGKPY	YITENYR
F8L-P33065 (166)	YFKYLGTTI	NHSADAVWI	FPTPINIHSI	OLSKFRTLLS	SLSNHEGKPH	YITENYR
F8L-AAA60846 (166)	YFKYLGTTI	NHSADAVWI	FPTPINIHSI	QLSKFRTLLS	SLSNHEGKPH	YITENYR
Consensus (166)	YFTYLGTTI	NHSADAVWI	IFPTPINIHSI	QLSKFRTLLS	SSSNHDGKPH	YITENYR
00110011040 (100)						 Section 5
(221)	221	230	240	250	260	275
D8L-P04195 (221	NPYKLNDDT	QVYYSGEII	RAATTSPARE	NYFMRWLSDL	RETCFSYYQK	YIEENKT
D8L-AAA48107 (221	NPYKLNDDT	OVYYSGEIII	RAATTSPAREI	NYFMRWLSDL	RETCFSYYQK	YIEGNKT
E8L-P33065 (221	NPYKLNDDT	EVYYSGEII	RAATTSPAREI	NYFMRWLSDL	RETCFSYYQK	CYIEGNKT
F8I -AAA60846 (221) NPYKLNDDT	EVYYSGEII	RAATTSPAREI	NYFMRWLSDL	RETCFSYYQK	CYIEGNKT
Consensus (221	,) NPYKLNDDT	QVYYSGEII	RAATTSPARE	NYFMRWLSDL	RETCFSYYQ	CYLEGNKT
						Section 6
(276) 276	290	30			
D8L-P04195 (276) FAIIAIVFV	FILTALLFF	MSRRYSREKQ	N		
D8L-AAA48107 (276) FAIIAIVFV	FILTAILFL	MSRRYSREKQ:	N ·		
F8L-P33065 (276) FAIIAIVFV	YILTAILFL	MSRRYSREKQ!	N		
F8L-AAA60846 (276) FAIIAIVFV	YILTAILFL	MSRRYSREKQ!	N		
Consensus (276) FAIIAIVFV	FILTAILFL	MSRRYSREKQ:	N		
•						

										Section 1
	(1)	1	1,	0	20)	,30		40	54
B5R-JQ1799			VVTLL	CVLPA	VVYST	CTVPTM	INNAKL	TSTETS	FNDKQKVT	FTCDQGYHS
B5R-D42526	(1)	MKTIS	VVTLL	CVLPA	VVYST	CTVPTM	INNAKL	TSTETS	FNNNQKVT	FTCDQGYHS
B7R-NP 042219	(1)	MKTIS	VVTLL	CVLPA	VVYST	CTVPTM	INNAKL	TSTETS	FNDKQKVT	FTCDSGYYS
B7R-CAA49116	(1)	MKTIS	VVTLL	CVLPA	VVYST	CTVPTM	INNAKL	TSTETS	FNDKQKVT	FTCDSGYYS
B7R-G36855	(1)	MKTIS	SVVTLL	CVLPA	VVYST	CTVPTM	INNAKL	TSTETS	FNDKQKVT	FTCDSGYYS
B7R-2015436GX	(1)	MKTIS	SVVTLL	CVLPA	VVYST	CTVPTM	INNAKL	TSTETS	FNDKQKVT	FTCDSGYYS
Consensus	(1)	MKTIS	SVVTLL	CVLPA	VVYST	CTVPTM	INNAKL	TSTETS	FNDKQKVT	FTCDSGYYS
							 			— Section 2
	(55)	55	.60		70	.8	0	,90		108
B5R-JQ1799			VCETI	KWKYE	NPCKK	MCTVSI	YISEL	YNKPLY	EVNSTMTL	SCNGETKYF
B5R-D42526	(55)	SDPNA	AVCETI	KWKYE	NPCKK	MCTVSI	YISEL	YNKPLY	EVNSTMTL	SCNGETKYF
B7R-NP 042219	(55)	LDPNA	AVCETI	KWKYE	NPCKK	MCTVSI	YVSEL	YNKPLY	EVNAIITL	ICKDETKYF
B7R-CAA49116		LDPNA	AVCETI	KWKYE	NPCKK	MCTVSI	YVSEL	YNKPLY	EVNAIITL	ICKDETKYF
B7R-G36855		LDPNA	AVCETI	KWKYE	NPCKK	MCTVSI	YVSEL	YNKPLY	EVNAIITL	ICKDETKYF
B7R-2015436GX	(55)	LDPNA	AVCETI	KWKYE	NPCKK	MCTVSI	YVSEL	YNKPLY	EVNAIITL	ICKDETKYF
Consensus	(55)	LDPNA	AVCETI	KWKYE	NPCKK	MCTVSI	YVSEL	YNKPLY	EVNAIITL	ICKDETKYF
										Section 3
	(109)	109		,120		,130		140	150	162
B5R-JQ1799	(109)	RCEEI	KNGNTS	WNDTV	TCPNA	ECQPLO	LEHGS	CQPVKE	KYSFGEYM	TINCDVGYE
B5R-D42526	(109)	RCEE	KNGNTS	WNDTV	TCPNA	ECQPLO	LEHGS	CQPVKE	KYSFGEYM	TINCDVGYE
B7R-NP_042219										
B7R-CAA49116										
										TINCDVGYE
B7R-2015436GX	(109)	RCEE	KNGNTS	SWNDTV	TCPNA	ECQSLÇ	LDHGS	CQPVKE	KYSFGEHI	TINCDVGYE
Consensus	(109)	RCEE	KNGNTS	SWNDTV	TCPNA	ECQSL	LDHGS	CQPVKE	KYSFGEHI	TINCDVGYE
										Section 4
	(163)		.170		,180		190		200	216
										HLSCKSGFT
										HLSCKSGFI
B7R-NP_042219										
B7R-CAA49116										
										HLSCKSGFI
B7R-2015436GX										
Consensus	(163)	VIGA	SYITC	ranswn	VIPSC	QQKCD	PSLSN	GLISGS	TFSIGGVI	HLSCKSGFI
										—— Section 5
	(217)	217		230)	240		250	260	
										KDVVQYEQE
										KDVVQYEQE
B7R-NP_042219										
										KDVVQYEQE
										KDVVQYEQE
B7R-2015436G										
Consensus	s (217 _.) LTGS	PSSTC:	TDGKM	ILATLI	CIRSNI	SEFDPV	FDGPDD	EIDPSKPS	KDVVQYEQE

					Section 6
(271)	271	280	290	300	317
B5R-JQ1799 (271)			VIFLISVIV	LVCSCDKNNDQYKFH	KLLP
B5R-D42526 (271)	IESLEATYH	IIIVALTIMO	VIFLISVIV	LVCSCDKNNDQYKFH	KLLP
				LVCSCNKNNDQYKFH	
				LVCSCNKNNDQYKFH	
				LVCSCNKNNDQYKFH	
B7R-2015436GX (271)	IESLEATY	IIIVALTIMO	SVIFLISVIV	LVCSCNKNNDQYKFH	KLLL
Consensus (271)	IESLEATY	HITLAVIII	SVIFLISVIV	LVCSCNKNNDQYKFH	KLLL

FIGURE 7 CONTINUED

									Section 1
	(1)	1	,10		20	,30	.40		55
H5R-P07242	(1)	MAWSI	TNKADTS	SFTKMAE	IRAHLK	NSAENKDI	KNEDIFPEI	VIIPSTKI	PKTKRAT
H5R-E42514	(1)	MAWSI	TNKADTS	SFTKMAE	IRAHLK	NSAENKDI	KNEDIFPEL	VIIPSTK	PKTKRAT
I5R-P33062	(1)	MAWSI	TNKADTS	SFTKMAE	IRAHLR	NSAENKD	KNDDIFPEI	VIIPSTK	PKTKRAT
I5R-AAA60836	(1)	MAWSI	TNKADTS	SFTKMAE	IRAHLR	NSAENKDI	KNDDIFPEI	VIIPSTK	PKTKRAT
Consensus	(1)	MAWSI	TNKADTS	SFTKMAE	IRAHLK	NSAENKDI	KNDDIFPEI	VIIPSTK	PKTKRAT
									- Section 2
	(56)			,70	.81		.90	,100	110
H5R-P07242	(56)	TPRKP	AATKRST	KKEE	VEEE	VVIEEYHÇ	TTEKNSP-	SPGV	SDIVESV
H5R-E42514							TTEKNSP-		
I5R-P33062	(56)	TPRKP	AATKRST	KKDKEKE	EVEEEE	VVIEEYHÇ	OTTEENSPE	PSSSPGV	GNIVESV
15R-AAA60836	(56)	TPRKP	AATKRST	KKDKEKE	EV-EEE	VVIEEYH	QTTEENSPE	PSSSPGV(GDIVESV
Consensus	(56)	TPRKP	AATKRSI	KKDKEKE	EVVEEE	VVIEEYH	QTTEKNSP	PSSSPGV	GDIVESV
									- Section 3
	(111)		120		130	.140	.15		165
							NHSARSDLS		
H5R-E42514	(102)	AAVEL	DDSDGDD)	EPMV	QVEAGKVI	NHSARSDLS	DLKVATDI	MIAKDFK
							NHSARSDLS		
15R-AAA60836									
Consensus	(111)	TAVEL	DDSNGDD	DNDNDND	DNEPMV	QVEAGKVI	NHSARSDLS	DLKVATDI	
						· · · · · · · · · · · · · · · · · · ·			Section 4
	(166)			,180		90	200	210	220
H5R-P07242	(148)	KIITR	ISAVSTV	LEDVQAA	GISRQF	TSMTKAIT	TLSDLVTE	:GKSKVVRI	KKVKTCK
							TTLSDLVTE		
							TTLSDLVTE		
15R-AAA60836									
Consensus	(166)	KIITR	RISAVSTV	LEDVQAA	GISRQF	TSMTKAI	TLSDLVTE	GKSKVVRI	
									- Section 5

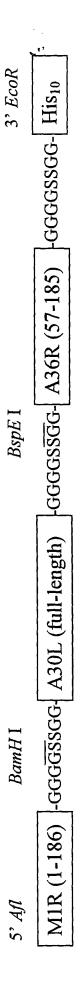
(221) 2221

H5R-P07242 (203) K

H5R-E42514 (203) K

I5R-P33062 (221) K I5R-AAA60836 (220) K Consensus (221) K

FIBURE 8



Schematic representation of LAA polyprotein construct. Respective coding regions for variola virus surface proteins M1R, A30L and A36R were first amplified with PCR using vaccinia virus genome as template. PCR-based mutagenesis was carried out to convert amino acid residues to those found in variola homologs. The coding sequence for LAA was assembled by in-frame ligation of three linker sequence. The 5' end of the construct has an Afl II restriction site and consensus Kozak sequence while a 10 histidine tag and components, MIR₁₋₁₈₆, A30L full-length and A36R₅₇₋₁₈₅, facilitated by the designed BamH I and BspE I restriction sites in the spacer-EcoR I site were engineered at the 3' end of the construct.